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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/604,779	08/15/2003	Matt Ewert	USF-196TCXC1	1778
23557	7590	07/24/2006	EXAMINER	
SALIWANCHIK LLOYD & SALIWANCHIK A PROFESSIONAL ASSOCIATION PO BOX 142950 GAINESVILLE, FL 32614-2950			PETERSEN, CLARK D	
			ART UNIT	PAPER NUMBER
			1655	

DATE MAILED: 07/24/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

<b>Office Action Summary</b>	<b>Application No.</b>	<b>Applicant(s)</b>
	10/604,779	EWERT ET AL.
	<b>Examiner</b>	<b>Art Unit</b>
	Clark D. Petersen	1655

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --  
**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

#### Status

- 1) Responsive to communication(s) filed on 15 August 2003.
- 2a) This action is FINAL.                    2b) This action is non-final.
- 3) Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

#### Disposition of Claims

- 4) Claim(s) 1-43 is/are pending in the application.
  - 4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) Claim(s) \_\_\_\_\_ is/are allowed.
- 6) Claim(s) 1-43 is/are rejected.
- 7) Claim(s) \_\_\_\_\_ is/are objected to.
- 8) Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

#### Application Papers

- 9) The specification is objected to by the Examiner.
- 10) The drawing(s) filed on \_\_\_\_\_ is/are: a) accepted or b) objected to by the Examiner.  
 Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
 Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

#### Priority under 35 U.S.C. § 119

- 12) Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
    - a) All    b) Some \* c) None of:
      1. Certified copies of the priority documents have been received.
      2. Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
      3. Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).
- \* See the attached detailed Office action for a list of the certified copies not received.

#### Attachment(s)

- |  |   |
|--|---|
| 1) <input checked="" type="checkbox"/> Notice of References Cited (PTO-892)  | 4) <input type="checkbox"/> Interview Summary (PTO-413)                     |
| 2) <input type="checkbox"/> Notice of Draftsperson's Patent Drawing Review (PTO-948)   | Paper No(s)/Mail Date. _____.   |
| 3) <input checked="" type="checkbox"/> Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)<br>Paper No(s)/Mail Date _____. | 5) <input type="checkbox"/> Notice of Informal Patent Application (PTO-152) |
|  | 6) <input type="checkbox"/> Other: _____.                                   |

## **DETAILED ACTION**

### ***Election/Restrictions***

Applicant's election without traverse of "bacteria" as the species of particle to be examined in the reply filed on April 17, 2006 is acknowledged.

Election was made **without** traverse in the reply filed on April 17, 2006.

Claims 1-43 were examined on their merits.

### ***Specification***

The disclosure is objected to because of the following informalities: The specification is objected to because the last paragraph ends with a comma and an incomplete sentence.

Appropriate correction is required.

### ***Claims***

Claims 16 and 17 are objected to because of the following informalities: the last line of claim 16 reads "incubating the sample for at room temperature". This phrase is nonsensical; it appears a parameter was left out after "for". Because claim 17 depends from claim 16, it, too, is objected to. Appropriate correction is required.

***Claim Rejections - 35 USC § 112***

The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 16-43 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 16 recites the limitation "the dried reagents". There is insufficient antecedent basis for this limitation in the claim.

Claims 16, 17, 18 and 19 recite the limitation "the solution". There is insufficient antecedent basis for this limitation in the claim.

Claim 17 recite the limitation "the dried reagent" (singular). There is insufficient antecedent basis for this limitation in the claim.

Claims 20-43 are rejected because they depend from claim 16.

***Claim Rejections - 35 USC § 102***

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 7, and 8 are rejected under 35 U.S.C. 102(b) as being anticipated by Zhang, et al (J Clin Microbiol, 1995). Zhang et al teach a method of collecting blood from patients. The blood is then broken up with buffer ATL which comprises SDS and

Proteinase K. The purified DNA is then subjected to PCR amplification, successfully showing the presence of *Streptococcus pneumoniae* DNA in patients' blood (see Materials and Methods, p. 597, see Results, p. 599; see Table 3, p. 600, as examples). Therefore the teachings of Zhang et al are deemed to anticipate the instant claims 1, 7, and 8.

Claims 1 and 2 are rejected under 35 U.S.C. 102(b) as being anticipated by Cassels et al (Biochem J, 1987). Cassels et al teach a method of adding plasminogen/streptokinase combination into an assay medium containing Tween 80, a detergent. They test the efficacy of the enzyme combination by measuring <sup>125</sup>I remaining in plasma, a form of blood sample. This is a measure of how much fibrin was cleaved by the streptokinase/fibrinogen (see Materials and Methods, p. 396, col. 2, for example); by measuring 125I-containing particles released, i.e., separating cleaved from noncleaved fibrin, Cassels et al could measure enzymatic activity. Therefore the teachings of Cassels et al are deemed to anticipate the instant claims 1 and 2.

#### ***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

- (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-6 are rejected under 35 U.S.C. 103(a) as being unpatentable over Cassels et al in view of Dupe et al (Thrombos Haemostas, 1981). The teachings of Cassels et al are described above and applied as before.

Additionally Cassels et al teach that streptokinase assays can be carried out in a buffer comprising potassium phosphate (see Materials and Methods, p. 396, for example).

Cassels et al do not specifically teach freezing and addition of salts in treating streptokinase/plasminogen mixtures.

Dupe et al teach that it is possible to freeze plasminogen which has been activated by streptokinase (i.e. the streptokinase has been added to the plasminogen), in a buffer comprising NaCl. This mixture is stored at -20 degrees (see Materials and Methods, p. 530, for example).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to freeze plasminogen and streptokinase together in a salt solution comprising NaCl, for use in a method of exposing blood to a streptokinase/ plasminogen mix, because Cassells et al teach that streptokinase and plasminogen are useful in extracting particles from blood samples, and Dupe et al teach that plasminogen and streptokinase can be stored together at -20 degrees. One would have been motivated to do so for the expected benefit that one could have activated plasminogen prepared ahead of a planned experiment.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Claims 1, 7, 8 and 9 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (J Clin Microbiol, 1995) in view of Heininger et al (J Med Microbiol, 2001). The teachings of Zhang et al are described above and applied as before.

Zhang et al do not teach the addition of DNase to blood samples.

Heininger et al teach that one can add recombinant DNase to blood samples that are also inoculated with bacterial DNA (see Materials and Methods, p. 244, for example). They teach that despite the addition of DNase, PCR is still possible to identify bacterial DNA in serum (see Materials and Methods, p. 244; see Results and Fig. 1 p. 245; see Discussion, p. 246, as examples).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use DNase in a method of purifying DNA from blood taught by Zhang et al, because Heininger et al teach that application of DNase to blood does not significantly inhibit its usefulness in PCR assays to detect bacterial DNA. One would have been motivated to do so for the expected benefit that DNase in serum can selectively degrade some DNA molecules; Heininger et al mention, for example, that other studies have demonstrated that serum DNases can degrade synthetic oligodeoxynucleotides (see Discussion, p. 247, for example).

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Claims 1, 7, 8, and 10 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (J Clin Microbiol, 1995) in view of Garg et al (Clin Chem, 1996). The teachings of Zhang et al are discussed above and applied as before.

Zhang et al do not teach addition of an endonuclease to blood samples.

Garg et al teach a method of isolating DNA from white blood cells in whole blood. In that method, they teach that lysate can be incubated with RNase, which is an endonuclease, to remove RNA which they describe as a contaminant, before further purifying DNA from the lysate. They then demonstrate that PCR can be successfully performed on the DNA isolated from RNase-treated blood lysate (see p. 647, col. 2 for example).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use RNase in a method of purifying DNA from blood taught by Zhang et al, because Garg et al teach that one can successfully add RNase to a blood lysate and subsequently perform PCR on the DNA. One would have been motivated to do so for the expected benefit of removing RNA which could be a potential contaminant.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Claims 1, 2, 11, and 12, 16, 17 and 23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (J Clin Microbiol, 1995) in view of Cassels et al (Biochem J, 1987) and Smith et al (Thromb Haemostas, 1982). The teachings of Cassels et al are discussed above and applied as before.

Additionally Cassels et al teach that the plasma clotting reaction in the presence of streptokinase/plasminogen can be performed at ambient temperature (20-24 degrees C; see "clot lysis assay", p. 396, col. 2, for example).

Cassels et al do not expressly teach the freeze-drying of streptokinase/plasminogen mixtures, and their dispensation into vials.

Smith et al teach that one can mix streptokinase with plasminogen and freeze dry it into vials (see Materials and Methods, "Fibrinolytic agents (III)", p. 269 col. 2, for example). They then teach that one can reconstitute it and use it to lyse fragments from fibrin clots in vivo in rabbits (see Materials and Methods, p. 269, for example). Additionally Smith et al teach that it is desirable to reconstitute freeze-dried enzymes in a solution before addition to blood, i.e. rabbits were dosed with 0.1 ml of streptokinase/plasminogen combination, thus it is inherent that a liquid, not a freeze-dried solid, was used (see Materials and Methods, p. 269 col. 1, for example).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to perform the assay of liberating particles from blood taught by Cassels et al and Zhang et al at room temperature, because Cassels et al teach that the

enzymes are effective at room temperature. It would have been obvious to freeze dry streptokinase/plasminogen mix in a method of purifying particles from blood taught by Cassels et al, because Smith et al teach that one can successfully freeze-dry streptokinase/plasminogen and then reconstitute it without losing enzymatic activity, and it would have been obvious to resuspend the freeze-dried enzyme composition in a method of liberating particles from blood taught by Cassels et al and Zhang et al, because Smith et al teach that it is possible to freeze-dry enzyme mixtures and subsequently add aqueous solution to them before use. One would have been motivated to perform the experiment at room temperature for the expected benefit that the enzyme composition would be more easily performed at room temperature without temperature-controlling devices, and one would have been motivated to freeze dry the enzymes for the expected benefit of making storage of the enzyme mix easier, and one would have put it in disposable vials to prevent the need of repeatedly freeze-drying and reconstituting the enzyme; additionally one would reconstitute the freeze-dried solid in aqueous solution because it would be more homogeneously applied than if applied as a solid.

Regarding claim 17, it would have been obvious to arrive at the concentrations as claimed through routine optimization.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Claims 1, 2, 11, and 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (J Clin Microbiol, 1995) in view of Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982), Garg et al (Clin Chem, 1996), Heininger et al (J Med Microbiol, 2001), Benjamin et al (Yeast, 1998) and Grotendorst et al (Toxicon, 1997). The teachings of Zhang et al, Cassels et al, Smith et al, Garg et al, and Heininger et al are discussed above and applied as before.

These authors do not specifically teach the addition of lipase and phospholipase A2 (PLA2) in addition to both DNase and endonuclease in a method of purifying particles from whole blood.

Benjamin et al teach that lipase is useful in making clinical diagnoses from blood because lipases hydrolyze lipids in blood samples creating measurable metabolites that allow for diagnoses of medical conditions (see Biosensors, p. 1080 col. 2, for example). Grotendorst et al teach that phospholipase A2 has hemolytic properties, and thus is useful for removing cells from blood (see Materials and Methods, p. 1782, for example).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to include phospholipase A2 and lipase in a method of purifying particles from blood taught by Zhang et al and Cassels et al, because Zhang et al and Cassels et al teach that it is possible to liberate particles from a blood product, and that enzymes are essential to that process, and Benjamin et al teach that lipase can be useful to add to a blood product for help in diagnosis of a medical condition, and Grotendorst et al teach that PLA2 can help dissolve blood components which is helpful in isolating particles of interest. One would have been motivated to do so for the

expected benefit of having another method for diagnosing a condition in a patient, in the case of lipase, and also for the expected benefit of further dissolving undesirable blood components in the case of PLA2.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Claims 1, 2, 11, and 13-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (J Clin Microbiol, 1995) in view of Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982), Garg et al (Clin Chem, 1996), Heininger et al (J Med Microbiol, 2001), Benjamin et al (Yeast, 1998), Grotendorst et al (Toxicon, 1997), and Kreilgaard et al (1998). The teachings of Zhang et al (J Clin Microbiol, 1995), Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982), Garg et al (Clin Chem, 1996), Heininger et al (J Med Microbiol, 2001), Benjamin et al (Yeast, 1998), Grotendorst et al (Toxicon, 1997) are discussed above and applied as before.

None of these references expressly teaches the addition of trehalose as a cryoprotectant during freeze drying of enzymes.

Kreilgaard et al teach that proteins have poor stability in aqueous solution. They further state that this problem can be overcome by freeze-drying proteins; however in this process, proteins often aggregate and lose their enzymatic activity. Therefore, trehalose can be added to a protein before the freeze drying process and that trehalose

affords protection to enzymes during freeze drying and storage as a dried solid (see, Introduction, p. 121, col. 2, for example).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to include trehalose in a method of freeze drying an enzymatic combination taught by Smith et al (1982) et al because Smith et al teach that it is possible to freeze dry enzymes in separate vials that are to be used later in a process of liberating particles from blood, and Kreilgaard et al teach that trehalose is a useful sugar to add to an enzyme composition before freeze-drying. One would have been motivated to do so for the expected benefit the enzyme composition for liberating particles from blood would be better protected and therefore more active.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Claims 1, 2, 11, 16, and 18-22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (J Clin Microbiol, 1995) in view of Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982), and Diez et al (J Biochem Biophys Methods, 1999). The teachings of Zhang et al (J Clin Microbiol, 1995), Cassels et al

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(Biochem J, 1987), and Smith et al (Thromb Haemostas, 1982) are discussed above and applied as before.

Additionally, Zhang et al teach that blood samples can be lysed using glass beads and vortexing, and then centrifuged to remove gross blood byproducts before the supernatant is decanted and processing continued with a Qiagen blood mini amp kit (see Materials and Methods, p. 597, for example). The Qiagen blood mini amp kit teaches the use of proteinase K and SDS as components of the process of purifying DNA from blood samples (see Qiagen DNA blood mini kit handbook, p. 12, for example).

None of the above references expressly teaches the use of endonuclease inactivation, DNase inactivation, or addition of aurintricarboxylic acid or sodium citrate to the sample.

Hallick et al teach that aurintricarboxylic acid (ATA) is a general nuclease inhibitor (see Introduction, p. 3055, for example). They demonstrate that addition of ATA to a nuclease reaction inhibits the reaction (see Figs 1 and 2, p. 3058, for example). Additionally they suggest that it would be useful to add ATA to prevent degradation of nucleic acids during nucleic acid isolation (see Introduction, p. 3055, for example). Von Pape et al (Thromb Res, 2000) teach that addition of sodium citrate has anticoagulant properties and that its addition to a blood sample has anticoagulant properties (see Discussion, p. 298, col. 2, for example).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to add ATA and sodium citrate when performing the assay of

liberating particles, for example RNA or DNA from blood taught by Cassels et al and Zhang et al, because Zhang et al teach that one can perform PCR from purified DNA, and Hallick et al teach that addition of ATA inhibits nucleases and suggest its usefulness when one desires to purify DNA. Additionally von Pape et al teach that sodium citrate has anticoagulant properties and therefore would keep blood from solidifying. One would have been motivated to do so for the expected benefit that using ATA and sodium citrate would allow one to keep blood liquefied, and ultimately isolate higher quality DNA.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Claims 1, 2, 11, 16, 23, and 24 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (J Clin Microbiol, 1995) in view of Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982), and Diez et al (J Biochem Biophys Methods, 1999). The teachings of Zhang et al (J Clin Microbiol, 1995), Cassels et al (Biochem J, 1987), and Smith et al (Thromb Haemostas, 1982) are discussed above and applied as before.

None of the above references expressly teaches DNase inactivation.

Sanyal et al (Mol Biotechnol, 1997) teach that one of skill in the art can properly perform PCR from RNA, i.e. remove contaminating DNA by adding DNase I in sample preparation, followed by inactivating the DNase I by heat treating the sample at 65

degrees C in the presence of EDTA. This procedure ensures that a sample contains only RNA and that no PCR signal will be generated by contaminating DNA.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to inactivate DNase when performing the assay of liberating particles from blood taught by Cassels et al and Zhang et al, because Cassels et al and Zhang et al teach that one can perform PCR from purified blood particles, and Sanyal et al teach that said particles can be RNA, and the best way to ensure a clean signal is to add DNase I to RNA-containing sample and then inactivate the DNase I before performing PCR. One would have been motivated to do so for the expected benefit that using DNase I, followed by inactivating it, would generate a signal from only RNA and not from contaminating DNA.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Claims 1, 2, 11, 16, 23, and 25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (J Clin Microbiol, 1995), in view of Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982), and Hughes et al (J Microbiol Methods, 2001). The teachings of Zhang et al (J Clin Microbiol, 1995), Cassels et al (Biochem J, 1987), and Smith et al (Thromb Haemostas, 1982) are discussed above and applied as before.

None of the above references expressly teaches endonuclease inactivation.

Diez et al teach that one can recover mostly intact RNA by treating cells with DEPC, a chemical that inhibits RNase (an endonuclease) and that this is effective in producing a sample that can be used with PCR for generating clean signals (see Abstract, p. 69, for example).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to inactivate RNase when performing the assay of liberating particles from blood taught by Cassels et al and Zhang et al, because Cassels et al and Zhang et al teach that one can perform PCR from purified blood particles, and Hughes et al teach that said particles can be RNA, and that an effective way to purify high-quality RNA is to add the RNase inhibitor DEPC to a sample before PCR is performed. One would have been motivated to do so for the expected benefit that using RNase one would generate a signal from only RNA and not from contaminating DNA.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Claims 1, 2, 11, 16, and 27-30 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (J Clin Microbiol, 1995), in view of Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982), and Semple, et al (Bioorg and Medicin Chem Lett, 2000). The teachings of Zhang et al (J Clin Microbiol, 1995), Cassels et al (Biochem J, 1987), and Smith et al (Thromb Haemostas, 1982) are discussed above and applied as before.

Additionally, the Qiamp blood mini kit handbook teaches that the process of purifying DNA according to their instructions is to filter lysate through a column by centrifugation and washing the filter with a wash buffer.

None of the above references expressly teach the use of sucrose or ecotine combined with HEPES as wash buffers.

A sucrose solution in HEPES is commonly known to one of ordinary skill in the art in purifying various biological molecules by centrifugation.

Semple et al teach that the protease inhibitor ecotin (also referred to as ecotine) is a powerful anticoagulant produced by some varieties of *E. coli*; it inhibits portions of the protease cascade that leads to fibrin clot formation (see Abstract; see Introduction; see Inhibitor Design Strategy, p. 2305). Further, they suggest that ecotin's activity could be useful in various applications in preventing thrombosis, i.e. clot formation (see, Abstract, p. 2305, for example).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use sucrose and ecotine washes of pellets when performing the assay of liberating particles from blood taught by Cassels et al and Zhang et al, because Cassels et al and Zhang et al teach that one can perform PCR from purified blood particles, and one of ordinary skill in the art knows that sucrose and HEPES can be useful as washing agents of purified nucleic acids before PCR is performed. It would have been obvious to use an ecotine HEPES wash because Semple et al teach that it is a powerful anticoagulant and would prevent accretion of undesirable coagulated blood proteins in the desired nucleic acid pellet. One would have been motivated to do so for

the expected benefit that using ecotine and sucrose in a HEPES buffer would yield a better-purified nucleic acid pellet.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Claims 1, 2, 11, 16, 31 and 32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (J Clin Microbiol, 1995), in view of Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982), Wang et al (2001) and Wang et al (FEBS Lett, 2000). The teachings of Zhang et al (J Clin Microbiol, 1995), Cassels et al (Biochem J, 1987), and Smith et al (Thromb Haemostas, 1982) are discussed above and applied as before.

None of the above references specifically teach the use of a biosensor or mass spectrometry in a method of detecting particles from blood identifying pathogens.

Wang et al (2000) teach that an endogenous cannabinoid is often upregulated by macrophages in the course of septic shock, and thus it is a method of identifying those patients in septic shock (see Abstract, p. 73, and Materials and Methods pp.74-75, for example). They teach that HPLC is an effective way to measure the cannabinoids in serum (see Materials and Methods, pp. 74-75; see Fig. 6, p. 81, as examples). Wang et al (2001) further teach that a surface plasmon resonance (Biacore 2000) biosensor also allows for detection of the cannabinoid (see Materials and Methods, p. 152; see

Results, p. 154, as examples). They report that a biosensor such as the Biacore 2000 provides a viable alternative method for measuring metabolites in serum.

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use HPLC or a biosensor to measure components purified from blood when performing the assay taught by Cassels et al and Zhang et al, because Cassels et al and Zhang et al teach that one can isolate particles or chemicals from purified blood, and Wang et al (2000) teach that an effective method of detecting bacterial sepsis in human serum is to measure chemicals produced by macrophages. In their follow-up paper (Wang et al 2001) they demonstrate that a biosensor provides an effective alternative method to HPLC to measure the same cannabinoid. One would have been motivated to do so for the expected benefit that chemical measurement using HPLC provides a means to measure chemical changes associated with sepsis other than direct PCR of bacterial pathogens, and biosensors provide an effective alternative to HPLC.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Claims 1, 2, 11, 16, and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (J Clin Microbiol, 1995), in view of Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982), and Hallick et al (Nucleic Acids Res, 1977). The teachings of Zhang et al (J Clin Microbiol, 1995), Cassels et al

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(Biochem J, 1987), and Smith et al (Thromb Haemostas, 1982) are discussed above and applied as before.

Additionally Cassels et al (1987) teach that a clot lysis buffer can contain sodium phosphate and NaCl.

Magnesium chloride is a standard biological assay solution component, known to those of ordinary skill in the art.

None of the above references expressly teaches addition of aurintricarboxylic acid.

Hallick et al teach that aurintricarboxylic acid (ATA) is a general nuclease inhibitor (see Introduction, p. 3055, for example). They demonstrate that addition of ATA to a nuclease reaction inhibits the reaction (see Figs 1 and 2, p. 3058, for example). Additionally they suggest that it would be useful to add ATA to prevent degradation of nucleic acids during nucleic acid isolation (see Introduction, p. 3055, for example).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to add ATA when performing the assay of liberating particles, for example RNA or DNA from blood taught by Cassels et al and Zhang et al, because Zhang et al teach that one can perform PCR from purified DNA, and Hallick et al teach that addition of ATA inhibits nucleases and suggest its usefulness when one desires to purify DNA. One would have been motivated to do so for the expected benefit that using ATA would allow one to isolate higher quality DNA.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Claims 1, 2, 11, 16, 33, and 34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (J Clin Microbiol, 1995), in view of Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982), Hallick et al (Nucleic Acids Res, 1977) and Lee et al (J Clin Microbiol, 1998). The teachings of Zhang et al (J Clin Microbiol, 1995), Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982) and Hallick et al (Nucleic Acids Res, 1977) are discussed above and applied as before.

None of the above references expressly teaches addition of Triton X-100.

Lee et al teach that Triton X-100 can be added to clinical serum specimens in a process of isolating DNA that is to be used for PCR identification of a pathogenic bacteria (see Materials and Methods, p. 2889, for example), and demonstrate that it is indeed a part of an effective protocol (see Fig. 5, p. 2890, for example).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to add Triton X-100 when performing the assay of liberating particles, for example DNA from blood sample taught by Cassels et al and Zhang et al, because Zhang et al teach that one can perform PCR from DNA purified from blood, and Lee et al teach that addition of Triton X-100 is a useful additive for isolating DNA to be used for PCR from blood. One would have been motivated to do so for the expected

benefit that using Triton X-100 would be an effective alternative detergent in isolating DNA from blood.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Claims 1, 2, 11, 16, 33, and 35-38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (J Clin Microbiol, 1995), in view of Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982), Hallick et al (Nucleic Acids Res, 1977), Zhang et al (Clin Chim Acta, 1999), and Pierre et al (J Biol Chem, 1995). The teachings of Zhang et al (J Clin Microbiol, 1995), Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982) and Hallick et al (Nucleic Acids Res, 1977) are discussed above and applied as before.

None of the above references expressly teaches addition of saponin or methyl 6-O-(N-heptylcarbamoyl)- $\alpha$ -D-glucopyranoside (also known as HECAMEG).

Zhang et al (Clin Chim Acta, 1999) teach that steroidal saponins have properties of inhibiting platelet aggregation and have an effect on hemolysis (see Abstract, p. 79, for example). They demonstrate the effect that these saponins have (see Fig. 4 and Table 1, p. 86, for example) and suggest that they could have use in antithrombotic, i.e. clot lysis, applications.

Pierre et al teach that HECAMEG is a useful detergent to add when solubilizing membranes, and in fact has been used specifically to isolate mycoplasma bacterial surface antigens (see Discussion, p. 29348, col. 1, for example).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to add saponins when performing the assay of liberating particles, for example DNA from blood sample taught by Cassels et al and Zhang et al, because Zhang et al teach that isolation of particles from blood is desirable when attempting to detect pathogens, and Zhang et al (Clin Chim Acta, 1999) teach that saponins have hemolytic and antithrombotic properties that break up particles from blood. It would have been obvious to one of ordinary skill in the art at the time the invention was made to add HECAMEG when performing the assay of liberating particles, for example bacterial surface antigens from blood samples taught by Cassels et al and Zhang et al, because Zhang et al teach that isolation of particles from blood is desirable when attempting to detect pathogens, and Pierre et al teach that HECAMEG allows for successful recovery of mycoplasma bacterial surface antigen particles. One would have been motivated to add saponins to blood samples for the expected benefit of breaking up larger particles in the blood such as erythrocytes and fibrin clots, making it easier to centrifuge DNA into a pellet without other contaminating blood products. One would have been motivated to add HECAMEG because HECAMEG is a useful detergent for isolating bacterial surface antigen particles, which would be useful when attempting to detect the presence of bacteria in a patient's blood.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

Claims 1, 2, 11, 16, 33, and 35-43 are rejected under 35 U.S.C. 103(a) as being unpatentable over Zhang et al (J Clin Microbiol, 1995), in view of Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982), Hallick et al (Nucleic Acids Res, 1977), Zhang et al (Clin Chim Acta, 1999), Lee et al (J Clin Microbiol, 1998) and Pierre et al (J Biol Chem, 1995). The teachings of Zhang et al (J Clin Microbiol, 1995), Cassels et al (Biochem J, 1987), Smith et al (Thromb Haemostas, 1982), Lee et al (J Clin Microbiol, 1998) and Hallick et al (Nucleic Acids Res, 1977) are discussed above and applied as before.

None of the above references expressly teaches addition of DTT, saponin or methyl 6-O-(N-heptylcarbamoyl)- $\alpha$ -D-glucopyranoside (also known as HECAMEG).

DTT is well known to one of ordinary skill in the art for reducing inappropriate cysteine bonds in proteins, preventing their aggregation and enzyme inactivation.

Zhang et al (Clin Chim Acta, 1999) teach that steroidal saponins have properties of inhibiting platelet aggregation and have an effect on hemolysis (see Abstract, p. 79, for example). They demonstrate the effect that these saponins have (see Fig. 4 and Table 1, p. 86, for example) and suggest that they could have use in antithrombotic, i.e. clot lysis, applications.

Pierre et al teach that HECAMEG is a useful detergent to add when solubilizing membranes, and in fact has been used specifically to isolate mycoplasma bacterial surface antigens (see Discussion, p. 29348, col. 1, for example).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to add saponins when performing the assay of liberating particles, for example DNA from blood sample taught by Cassels et al and Zhang et al, because Zhang et al teach that isolation of particles from blood is desirable when attempting to detect pathogens, and Zhang et al (Clin Chim Acta, 1999) teach that saponins have hemolytic and antithrombotic properties that break up particles from blood. It would have been obvious to one of ordinary skill in the art at the time the invention was made to add HECAMEG when performing the assay of liberating particles, for example bacterial surface antigens from blood samples taught by Cassels et al and Zhang et al, because Zhang et al teach that isolation of particles from blood is desirable when attempting to detect pathogens, and Pierre et al teach that HECAMEG allows for successful recovery of mycoplasma bacterial surface antigen particles. One would have been motivated to add saponins to blood samples for the expected benefit of breaking up larger particles in the blood such as erythrocytes and fibrin clots, making it easier to centrifuge DNA into a pellet without other contaminating blood products. One would have been motivated to add HECAMEG because HECAMEG is a useful detergent for isolating bacterial surface antigen particles, which would be useful when attempting to detect the presence of bacteria in a patient's blood.

Regarding the specific values for amounts of added reagents, these can be determined through result-effective adjustment.

Based upon the teachings of the cited references, the level of skill of one of ordinary skill in the art, and absent any evidence to the contrary, one would have a reasonable expectation of success in practicing the claimed invention.

***Conclusion***

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Clark D. Petersen whose telephone number is (571)272-5358. The examiner can normally be reached on M-F 8:30-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey can be reached on (571)272-0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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CDP  
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